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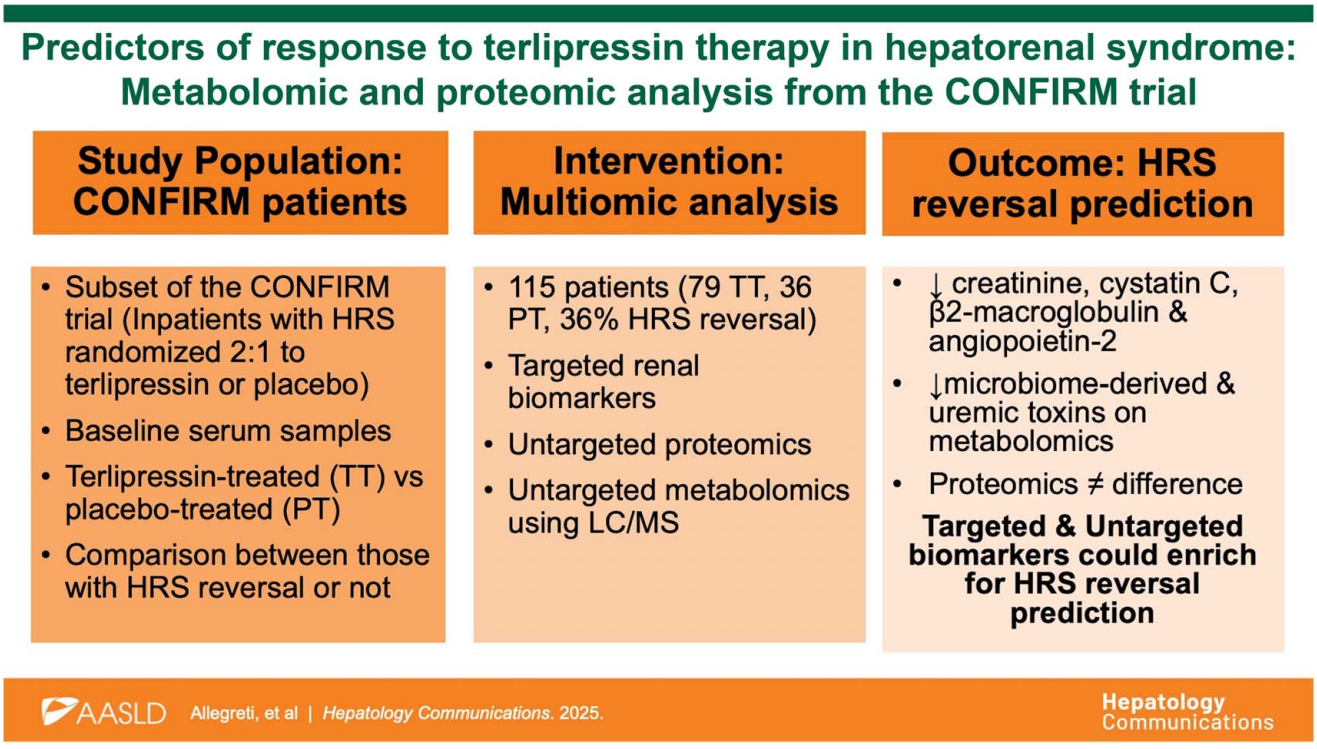
VISUAL ABSTRACT



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ORIGINAL ARTICLE

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Predictors of response to terlipressin therapy in hepatorenal syndrome: Metabolomic and proteomic analysis from the CONFIRM trial

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Abstract

Background: Terlipressin is the only FDA-approved vasoconstrictor for hepatorenal syndrome (HRS). The CONFIRM study is the largest trial of terlipressin versus placebo. Novel predictors of HRS response are required to enrich patient selection and optimize outcomes.

Methods: Samples at treatment initiation were tested using (a) liquid chromatography–mass spectrometry of 1594 plasma/1420 urine metabolites (Metabolon Inc.), (b) aptamer-based array of 7289 plasma proteins (SomaScan), and (c) 14 plasma/urine pre-specified assays. The CONFIRM trial's original definition of HRS response [2 serum creatinine (SCr) <1.5 mg/dL separated by >2 h] was used as the primary outcome.

Results: In all, 115 patients [79 terlipressin-treated (TT) and 36 placebo-treated (PT)] provided samples. Baseline characteristics, outcomes, and 2:1 TT:PT allocation were preserved from the original 300-patient trial. A total of 36 out of 116 (31.0%) patients achieved HRS reversal. HRS reversal was associated with lower SCr ($p=0.001$), cystatin C ($p=0.005$), angiopoietin-2 ($p=0.04$), and beta-2 microglobulin ($p=0.006$). In metabolite analysis, PT had the most significant differences in HRS reversal [n = 26 plasma, n = 50 urine, including lower urine levels of those centered on sulfated secondary bile acids (microbiome-derived), N-acetylated amino acids, catechols (both uremic toxins), and phosphocholines (cell membrane integrity)], with fewer in TT (n = 1 plasma, n = 2 urine), and in all patients (n = 3 plasma, n = 7 urine). There were no significant aptamers associated with HRS reversal after false-discovery correction.

Abbreviations: AKI, acute kidney injury; FDR, false discovery rate; HRS, hepatorenal syndrome; i.v., intravenous; IL-18, interleukin-18; IRB, institutional review board; KIM-1, kidney injury molecule-1; LC–MS, liquid chromatography–mass spectrometry; L-FABP, liver fatty acid binding protein; MAP, mean arterial pressure; NGAL, neutrophil gelatinase–associated lipocalin; PCA, principal component analysis; PT, placebo-treated; RFU, relative frequency unit; RSD, relative standard deviation; SCr, serum creatinine; TFF-3, trefoil factor-3; TIMP-1, tissue inhibitor of metalloproteinases-1; TT, terlipressin-treated.

Josh Levitsky: deceased status.

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Conclusions: SCr, cystatin C, angiopoietin-2, and beta-2 microglobulin were associated with HRS reversal. Protein and metabolite signals centered on microbiome function and uremic toxins appeared more robust in PT patients, likely selecting a subgroup that may recover without terlipressin. Use of novel biomarkers may enrich for terlipressin response.

Keywords: acute kidney injury, acute on chronic liver failure, cirrhosis, liver failure, liver transplant, renal failure, vasoconstrictor

INTRODUCTION

Terlipressin is a V1a receptor agonist aimed at restoring renal perfusion through splanchnic vasoconstriction and reduction of portal hypertension.[1] Along with intravenous (i.v.) albumin, it is recommended as first-line therapy for hepatorenal syndrome (HRS) in multiple guidelines and is the only FDA-approved vasoconstrictor for this condition.[2–6] HRS represents a heterogenous and severe renal impairment, and despite optimal medical management, HRS reversal is seen only in a minority of patients treated with terlipressin.[7–10] Therefore, it is vital to identify new markers that are predictive of response to therapy in order to enrich patient selection and optimize outcomes.

The CONFIRM trial was the largest randomized trial comparing terlipressin versus placebo (with both arms receiving i.v. albumin) for the treatment of HRS. One of the prespecified exploratory aims of this trial was to identify novel research biomarkers that predicted response to terlipressin. Several categories of biomarkers have been posited as potential areas of interest, including markers of tubular function/damage, perfusion/circulation, inflammation, and microbiome-derived bile acids.[11,12] Therefore, using blood and urine samples collected from patients at the time of treatment initiation in the CONFIRM trial, we performed 3 types of sample analysis to identify predictors of HRS response.

METHODS

Study population and setting

The CONFIRM trial was a phase 3, North American, double-blinded, randomized, placebo-controlled trial comparing terlipressin versus placebo plus IV albumin for the treatment of HRS (NCT02770716). The full trial protocol is described elsewhere,[8] but in brief, following a screening period where each patient received a 48-hour IV albumin challenge, patients with HRS who met entry criteria were randomized 2:1 to terlipressin treatment (TT) or placebo treatment (PT). Treatment continued for 24 hours after serum creatinine (SCr) was below 1.5 mg/dL for 2 consecutive measures (defined as HRS reversal), or other stopping criteria were met (no improvement by day 4, patient required renal replacement therapy, liver transplantation, TIPS, or received 14 days of treatment). Patients who received midodrine/octreotide were eligible for enrollment if these medications were stopped prior to randomization.

The trial was IRB approved at all sites and participants gave informed consent. Patients who consented to the primary trial were asked to provide additional consent to store blood and urine from the time of treatment initiation for future biomarker analysis. Patients who provided this consent and had samples available represented the population of this subanalysis. Clinical data, demographics, and outcomes were taken from the safety data set of the CONFIRM trial.

Outcomes and statistical analysis

The primary outcome of this analysis was HRS reversal, as defined above. Three types of biomarker analysis were performed: (1) liquid chromatography–mass spectrometry (LC–MS) of 1594 plasma/1420 urine metabolites (Metabolon Inc.), (2) aptamer-based array of 7289 plasma proteins (SomaScan), and (3) 14 a priori serum/urine ELISA selected from prior literature around HRS and acute kidney injury (AKI) in cirrhosis, including serum cystatin C, liver fatty acid binding protein (L-FABP), interleukin 18 (IL-18), kidney injury molecule-1 (KIM-1), tissue inhibitor of metalloproteinases-1 (TIMP-1), angiopoietin-2, osteopontin, VEGF, beta-2 microglobulin, trefoil factor-3 (TFF-3), as well as urine sodium, urea, protein/creatinine ratio, and neutrophil gelatinase–associated lipocalin (NGAL).[13–18] Analytes were measured against the primary outcome for all patients and restricted to TT or PT patients.

Metabolomic data analyzed included amino acids, carbohydrates, lipids, nucleotides, microbiota metabolism, energy, cofactors/vitamins, xenobiotics, and novel/unnamed metabolites using methodology similar to previous studies in cirrhosis.[19,20] To account for variability related to patient-level variables, ANCOVA

analyses were performed, adjusting for false discovery, represented by the *q* value generated by the method of Storey and Tibshirani. Following log transformation and imputation of missing values, if any, with the minimum observed value for each compound, ANOVA contrasts and Welch 2-sample *t* test were used to identify biochemicals that differed significantly between groups. Finally, ANCOVA was performed, controlling for age, sex, MELD, and eGFR at study entry. An estimate of the false discovery rate (FDR) was calculated to consider the multiple comparisons that normally occur in metabolomic-based studies.[21] Instrument variability was determined by calculating the median relative standard deviation (RSD) for the internal standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (ie, noninstrument standards) present in 100% of the Client Matrix samples, which are technical replicates of pooled client samples. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (ie, noninstrument standards) present in the technical replicates. Metabolites that were independently associated with the outcome of interest, HRS reversal.

Similarly, proteomic analysis adjusted for false discovery, represented by the *q* value generated by the method of Storey and Tibshirani. Analytes were measured by Somalogic using standard methodologies of preparing/labeling aptamers using a photocleavable linker, and measuring fluorescence intensity in relative fluorescence units (RFU) on a microarray chip.[22] Principal component analysis (PCA) was applied to aptamers to reduce multidimensional data into fewer dimensions (principal components), improving analysis by highlighting significant patterns.

For continuous clinical variables and biomarkers measured by ELISA, association with HRS reversal was assessed using Student *t* test or Wilcoxon rank sum test and displayed as mean (SD) or median (interquartile range), depending on distribution. Categorical variables were analyzed using chi-square or the Fisher exact test. Two-tailed *p* values < 0.05 were considered statistically significant. Analysis was performed using R Studio version 4.1 (Vienna, Austria).

Ethics

The CONFIRM trial protocol was approved by each participating site's institutional review board (IRB). This substudy was also approved by the IRB at the corresponding author's institution. Each site abides by the guidelines set forth by the Declaration of Helsinki and Istanbul. Participants who provided samples for this substudy provided specific informed consent for biomarker analysis.

RESULTS

Patient demographics and clinical characteristics

Of the 300 patients originally enrolled in the CONFIRM trial (199 TT and 101 PT), 115 patients (79 TT and 36 PT) consented to provide samples for analysis. All 115 patients provided blood samples, and 89 provided urine samples. Baseline characteristics, outcomes, and the 2:1

TABLE 1 Demographics and clinical characteristics by HRS reversal status

	All (n = 115)	HRS reversal (n = 36)	No reversal (n = 79)	*p*
Age, y [mean (SD)]	53.9 (12.03)	53.9 (12.95)	53.9 (11.67)	0.99
Male sex (%)	67 (58.3)	25 (69.4)	42 (53.2)	0.15
Alcohol-associated cirrhosis (%)	78 (67.8)	28 (77.8)	50 (63.3)	0.19
Mean arterial pressure (mm Hg)	77.0 [70.3, 85.8]	78.7 [72.1, 84.3]	76.7 [68.7, 86.0]	0.48
MELD score	32 [25,39]	31 [25, 39.25]	33 [26,39]	0.50
Child–Pugh score	10 [8,11]	10 [8.75, 12]	10.5 [8,11]	0.73
Serum albumin (g/dL)	3.7 [3.4, 4.1]	3.6 [3.1, 4.05]	3.7 [3.5, 4.2]	0.08
Serum sodium (mmol/L)	133.5 [130.25, 137.75]	134 [129.75, 137.25]	133 [131, 137.75]	0.94
Serum creatinine (mg/dL)	3.10 [2.60, 3.90]	2.75 [2.50, 3.30]	3.50 [2.65, 4.10]	0.001
Serum bilirubin (mg/dL)	5.45 [2.00, 23.22]	4.55 [1.98, 18.12]	5.65 [2.08, 25.00]	0.50
Serum international normalized ratio	2.00 [1.60, 2.53]	2.10 [1.52, 2.78]	2.00 [1.70, 2.50]	0.79
Mean arterial pressure—baseline	78.02 (11.75)	79.75 (10.54)	77.20 (12.27)	0.28
Mean arterial pressure—day 3	78.60 (11.35)	81.82 (11.17)	77.06 (11.18)	0.05
ACLF score ≥ 3	19 (16.5%)	6 (16.7%)	13 (16.5%)	0.99

Note: All values were taken at the time of enrollment. All continuous variables are presented as median [interquartile range] unless otherwise noted.
Abbreviations: ACLF, acute-on-chronic liver failure; HRS, hepatorenal syndrome.

TT:PT allocation were preserved relative to the original trial population. Among the 115 patients in this analysis, mean age was 59.3 (12.03) years, 67/115 (58.3%) were male, alcohol use disorder was the most common etiology of cirrhosis [78/115 (67.8%)], and median MELD score was 32 (IQR 25, 39). Furthermore, 19/115 (16.5%) of patients had a baseline ACLF score ≥ 3. 36/115 (31.3%) patients achieved the primary outcome of HRS reversal, including 31/79 (39.2%) TT patients and 5/36 (13.9%) PT patients ($p = 0.012$). Clinical variables and demographics are displayed in Table 1 by HRS reversal status. Of these variables, only lower SCr at randomization was associated with HRS reversal (2.75 [2.50, 3.30] vs. 3.50 [2.65, 4.10] mg/dL, $p = 0.001$). Similar results were noted when restricting to TT and PT patients, though SCr was not significantly associated with HRS reversal among PT patients in this small sample ($p = 0.14$), and all 5 PT patients who achieved HRS reversal were male. Mean arterial pressure (MAP) was statistically similar between groups at baseline but significantly increased in the HRS reversal group compared to the placebo group at day 3 (Table 1 and Supplemental Table S1, http://links.lww.com/HC9/C52).

ELISA biomarker analysis

Fourteen serum and urine biomarkers were measured to determine association with HRS reversal (Table 2). Among all patients, patients who achieved HRS reversal had lower serum cystatin C (3.39 [3.05, 4.21] vs. 4.02 [3.55, 4.88] g/dL, $p = 0.005$), serum angiopoietin-2 (12.13 [9.52, 19.77] vs. 17.10 [11.59, 25.48] ng/mL, $p = 0.04$), and serum beta-2 microglobulin (6.47 [4.43, 8.60] vs. 8.70 [5.89, 11.26] mg/L, $p = 0.006$). There were no statistically significant differences in serum L-FABP, IL-18, KIM-1, osteopontin, VEGF, and TFF-3, or urine sodium, urea, protein/creatinine ratio, and NGAL by HRS reversal status. Among TT patients, serum cystatin C and serum osteopontin were lower in the 31/79 (39%) patients who achieved HRS reversal ($p = 0.02$ for both). Among PT patients, serum VEGF was lower in the 5/31 (16%) patients who achieved HRS reversal ($p = 0.02$, Supplemental Table S2, http://links.lww.com/HC9/C52).

Metabolomic analysis

Urinary metabolomics

A total of 1420 urinary metabolites (1056 previously identified) were analyzed. After normalization for urine osmolality, 203 metabolites (50↓/153↑) were different between HRS reversal groups in the entire cohort. Among those who achieved HRS reversal, 48 urinary metabolites (4↓/44↑) were different between the PT and TT groups. Among PT patients, 92 urinary metabolites (10↓/82↑) were different between those who achieved HRS reversal versus not. Among TT patients, 115 urinary metabolites (64↑/51↑) were different between those who achieved HRS reversal versus not.

Plasma metabolomics

A total of 1594 plasma metabolites (1283 previously identified) were analyzed. For the entire cohort, 121 plasma metabolites (8↓/113↑) were different between

TABLE 2 Biomarker levels by HRS reversal status

	All (n = 115)	HRS reversal (n = 36)	No reversal (n = 79)	*p*
Serum cystatin C (mg/L)	3.90 [3.17, 4.67]	3.39 [3.05, 4.21]	4.02 [3.50, 4.88]	0.005
Serum L-FABP (ng/mL)	67 [0, 117]	67 [0, 108]	80 [0, 117]	0.54
Serum IL-18 (ng/L)	694 [485, 1160]	554 [366, 1044]	800 [513, 1160]	0.13
Serum KIM-1 (pg/mL)	29 [17, 52]	35 [18, 53]	28 [17, 52]	0.87
Serum TIMP-1 (ng/mL)	405 [315, 762]	370 [294, 490]	450 [324, 832]	0.08
Serum angiopoietin-2 (ng/mL)	16.58 [10.54, 23.87]	12.13 [9.52, 19.77]	17.10 [11.59, 25.48]	0.04
Serum osteopontin (ng/mL)	106.3 [59.6, 208.2]	95.0 [60.4, 143.0]	109.5 [61.5, 232.7]	0.20
Serum VEGF (pg/mL)	14.23 [9.05, 44.16]	12.20 [9.44, 34.97]	14.88 [8.92, 37.43]	0.65
Serum TFF-3 (ng/mL)	7.50 [7.50, 10.73]	7.50 [6.39, 10.95]	7.69 [7.50, 10.52]	0.42
Serum beta-2 microglobulin (mg/L)	7.66 [5.17, 10.21]	6.47 [4.43, 8.60]	8.70 [5.89, 11.26]	0.006
Urine sodium (mmol/L)	10.2 [7.3, 12.7]	9.6 [6.7, 11.8]	10.3 [7.9, 12.8.2]	0.32
Urine urea (mg/dL)	912 [673, 1191]	980 [782, 1179]	865 [644, 1193]	0.30
Urine protein/creatinine ratio (g/g)	0.10 [0.06, 0.16]	0.10 [0.06, 0.16]	0.10 [0.06, 0.16]	0.66
Urine NGAL (ng/mL)	75 [9, 258]	65 [6, 149]	92 [31, 283]	0.09

Note: Urine assays were available in 89 patients. All results reported as median [interquartile range].
Abbreviations: HRS, hepatorenal syndrome; KIM-1, kidney injury molecule-1; L-FABP, liver fatty acid binding protein; NGAL, neutrophil gelatinase–associated lipocalin; TFF-3, trefoil factor-3; TIMP-1, tissue inhibitor of metalloproteinases-1.

HRS reversal groups. Among those who achieved HRS reversal, 159 plasma metabolites (86↓/73↑) were different between the PT and TT groups. Among PT patients, 115 plasma metabolites (29↓/86↑) were different between those who achieved HRS reversal versus not. Among TT patients, 114 plasma metabolites (16↓/98↑) were different between those who achieved HRS reversal versus not.

On partial least squares discriminant analysis, there was a greater overlap between plasma and urine metabolites between HRS reversal groups among TT patients, whereas there was some separation noted among PT patients. ANCOVA analyses controlled for age, sex, eGFR, and MELD showed that several urinary and plasma metabolites were different between those who achieved HRS reversal or not (Supplemental Figure S1, http://links.lww.com/HC9/C52).

For the entire cohort, only 3 plasma and 7 urine metabolites showed significant differences between those who achieved HRS reversal versus not after correcting for false discovery (Figure 1). These included reduced urinary expression of 2 secondary bile acids (glycodeoxycholate 3-sulfate and glycoursodeoxycholic acid sulfate). Among TT patients, only 1 plasma and 2 urine metabolites showed significant differences between HRS reversal groups, including reduced urinary expression of the secondary bile acid glycodeoxycholate 3-sulfate. PT patients had the most significant differences between HRS reversal groups, including 26 plasma and 7 urine metabolites. These included lower levels of microbiome-derived sulfated secondary bile acids, potential uremic toxins (N-acetylated amino acids and catechols), and phosphocholines involved in cell membrane integrity in patients who achieved HRS reversal.

Proteomic analysis

PCA showed significant separation of aptamers in PT patients, but not among all patients or TT patients (Figure 2). Heat maps of aptamers that were differentially expressed by HRS reversal status for the entire cohort, TT patients, and PT patients are visualized in Figure 3. After correcting for false discovery, no proteins reached statistical significance among all patients or TT patients. However, among PT patients, heat shock factor 2 binding protein was higher in HRS responders vs. not (log-fold change 1.22, FDR-adj $p = 0.047$).

DISCUSSION

In this prespecified, exploratory analysis of enrollment samples from the CONFIRM trial, we provide the most comprehensive analysis of biomarkers to predict response to terlipressin to date. We have demonstrated that several novel markers are associated with HRS reversal and have identified pathways that warrant further exploration as contributing factors in the pathogenesis of HRS.

Like in previous studies, our study validated the finding treatment of HRS earlier during injury (evidence by lower SCr and cystatin C) levels is associated with greater chance of HRS reversal. There is increasing evidence that cystatin C may provide better estimation of kidney function and/or survival prognostication in cirrhosis.[23–25] However, its role in AKI is less clear, as unlike SCr, it lacks a validated staging system for AKI severity.[2,6,26] We suggest that both markers be routinely measured in this population.

Our study found lower levels of serum angiopoietin-2, important in the regulation of endothelial cell inflammation, vascular function, and hepatocyte repair, in patients who achieved HRS reversal. This finding suggests the potential importance of vascular biology and inflammation in the pathophysiology of HRS and treatment response.[15,27,28] Serum beta-2 microglobulin, another general marker of tubular function and glomerular filtration, was also lower in those who achieved HRS reversal. Even though this test lacks specificity, these results may emphasize that patients with intact nephrons (without tubular injury) are more likely to respond to restoration of renal perfusion.[14,29] This also supports that initiating HRS treatment at earlier stages of AKI is beneficial.

Analysis of metabolomic and proteomic profiles emphasized several pathways of interest that may contribute to either the pathogenesis of HRS or response to therapy. Metabolites derived from gut microbiota have been associated with poor outcomes in acute-on-chronic liver failure as well as AKI in cirrhosis.[19,20] In our study, lower serum concentration of several of these metabolites, including sulfated secondary bile acids, was associated with HRS reversal. Cirrhosis progression and kidney damage have been associated with bile acids even without obvious cholestasis, likely due to gut microbiome and immune-related changes.[30] Bile acid metabolism and its interaction with kidney function are complicated, encompassing both proinflammatory and anti-inflammatory effects, as well as the potential for direct tubular damage by way of cholemic or bile cast nephropathy; thus, it is difficult to fully delineate the mechanistic linkage in our analysis.[31–33] Metabolites related to uremia were also lower in those with HRS reversal, further supporting that patients with more advanced kidney failure are less likely to respond to treatment.[20]

Overall, there were relatively few analytes that were significantly different by HRS reversal status among the entire population and TT patients, while PT patients had more significant findings. This may suggest there is a subgroup of patients with HRS who may improve with supportive care and/or IV albumin therapy alone,

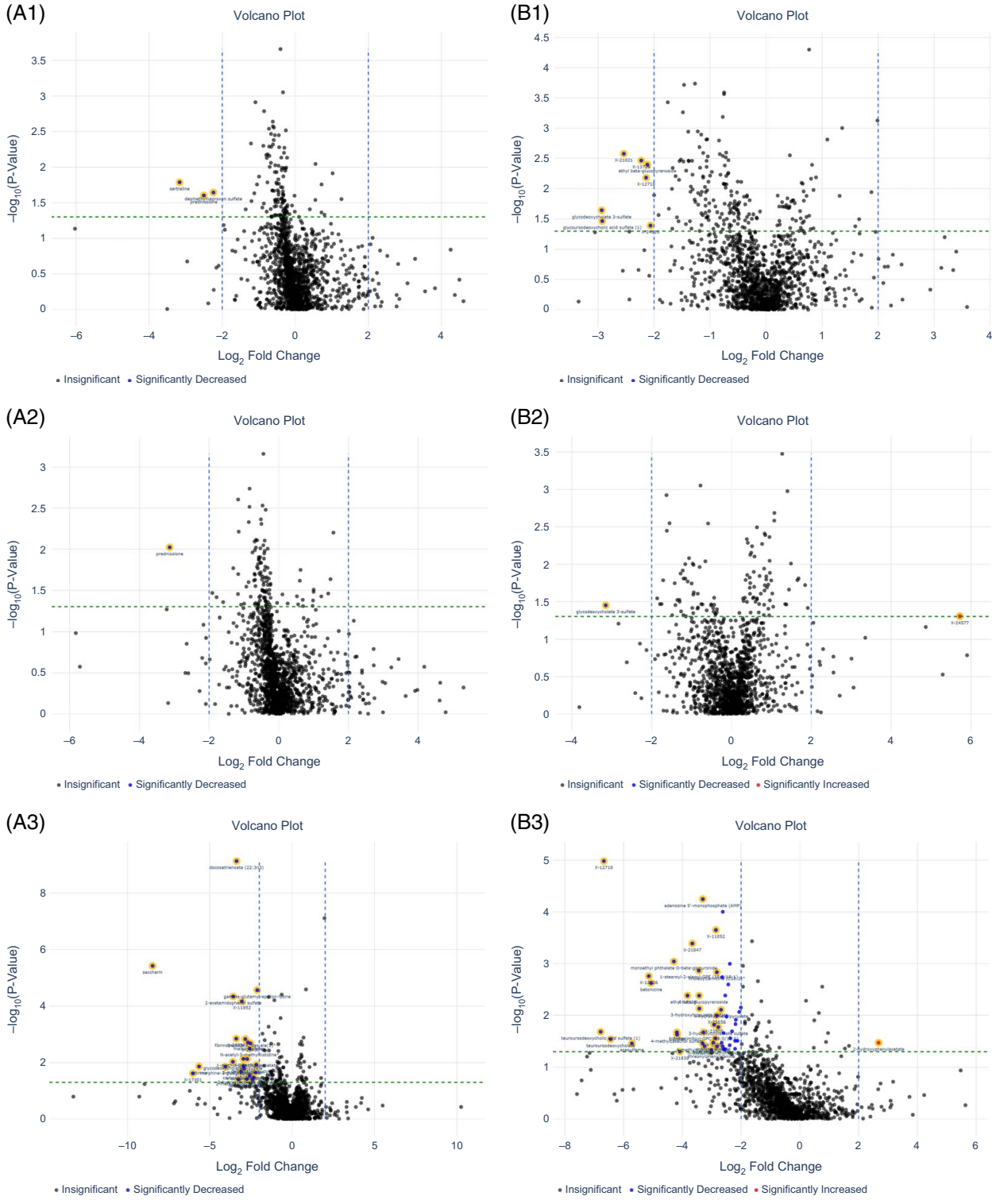

FIGURE 1 Volcano plots of plasma (left panels, A) and urine (right panels, B) metabolites that were differentially expressed in patients who achieved HRS reversal among (1) all patients, (2) terlipressin-treated patients, and (3) placebo-treated patients. Metabolites above the horizontal green line met the threshold for statistical significance after false discovery correction. Metabolites highlighted in orange were significantly lower in patients who achieved HRS reversal. A positive log-fold change represents increased expression in patients who achieved HRS reversal. Abbreviation: HRS, hepatorenal syndrome.

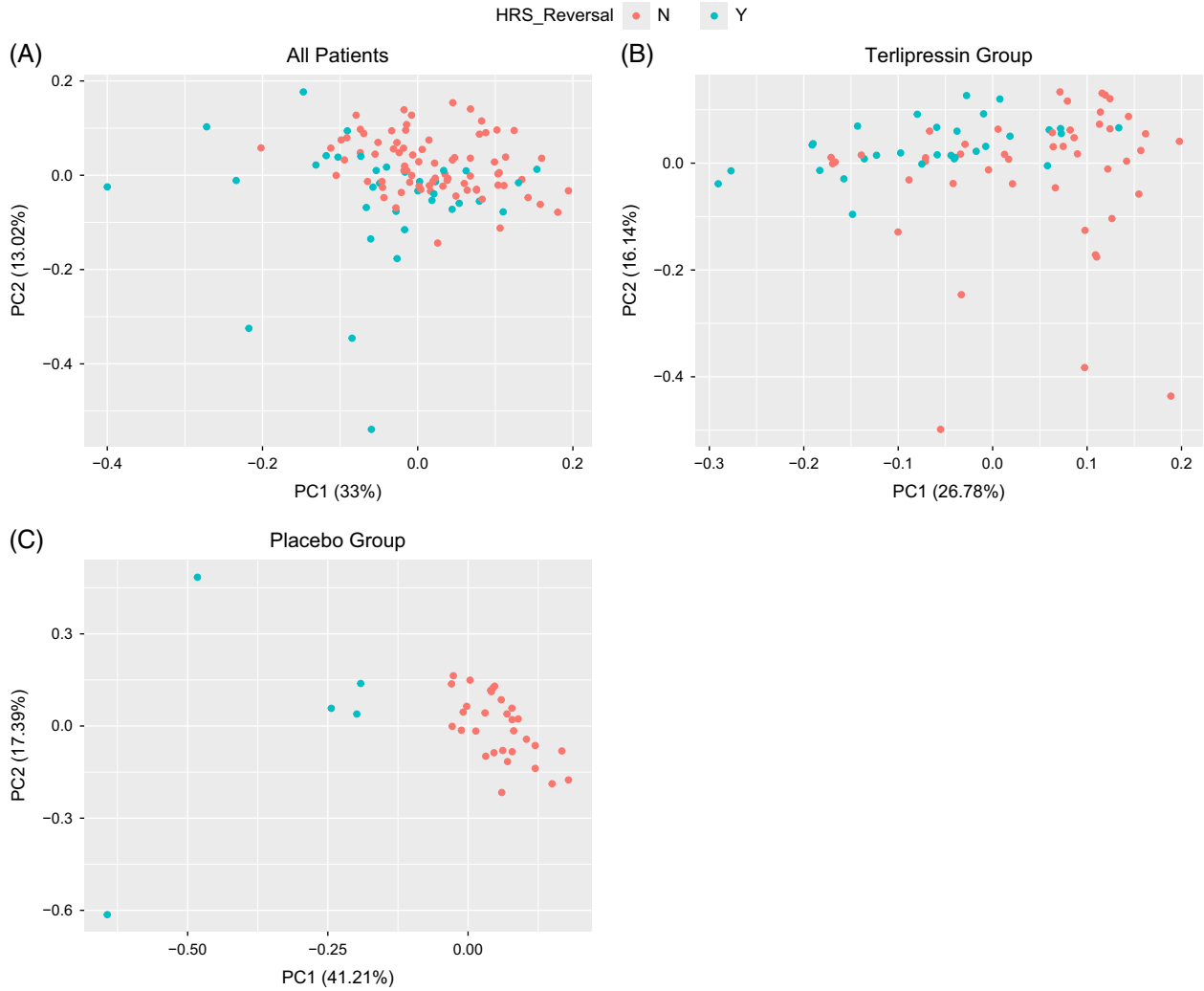

FIGURE 2 Principal component analysis of protein aptamers showing differential expression by HRS reversal status for (A) all patients, (B) terlipressin-treated patients, and (C) placebo-treated patients. Abbreviation: HRS, hepatorenal syndrome.

thereby reducing the risks associated with terlipressin exposure for this select group.[34] Such information could be used to supplement a thoughtful clinical assessment of terlipressin candidacy in high-risk subgroups, such as patients with higher MELD scores, in situations on the fringes of the current label for terlipressin, or where costs/health care resources are limited. Heat shock factor 2 binding protein, the only aptamer that was significantly increased among PT patients who achieved HRS reversal, is a chaperone protein that has been associated with tumor progression in lung adenocarcinoma and has a role in liver injury protection.[35,36] This report is the first description of HSP-2 and HRS, and further studies are needed to elucidate potential mechanisms.

Some negative findings are also worthy of discussion. While prior studies have shown that urine sodium and urinary NGAL can distinguish HRS from other forms of AKI, such as acute tubular necrosis, we did not see either emerge as predictors of HRS reversal in our results.[16,18,37] While this may indeed represent a true-negative signal, it is important to note that the CONFIRM trial included a carefully selected group of patients with HRS, resulting in a very low and narrow range of urinary sodium and NGAL. For example, this study's median urinary NGAL level of 75 ng/mL was far lower than clinically useful NGAL cut-points suggested in prior studies, which have been reported between 165 and 650 ng/mL.[38] We would also caution not to overinterpret negative findings in light of small sample size, especially for urine assays, where there were only 89 patients represented, thus limiting power. Further larger studies are needed to determine if this lack of observable difference was due to lower numbers.

These results should be viewed in the context of their limitations. While the CONFIRM trial's rigorous patient selection made this a unique and relatively homogenous HRS population, all biomarker analyses were exploratory in nature. Some biomarkers were shared across groups, while few were specific to the placebo

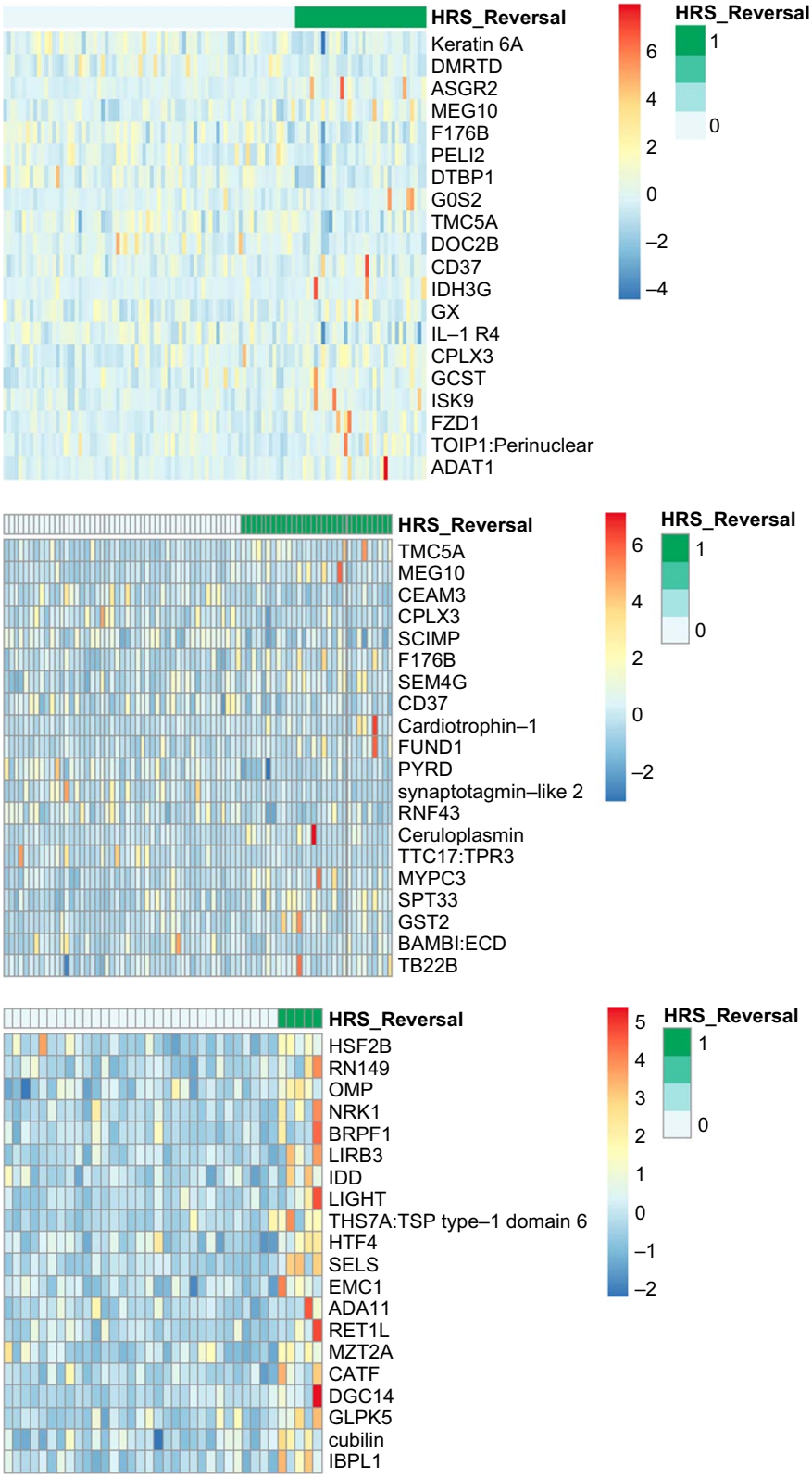

FIGURE 3 Heat maps demonstrating differential expression of protein aptamers between patients who achieved HRS reversal among all patients (top), terlipressin-treated patients (middle), and placebo-treated patients (bottom). Each cell represents an individual patient sample, with red noting increased expression and blue representing decreased expression relative to the inverse HRS reversal status. Abbreviation: HRS, hepatorenal syndrome.

group, which raises the possibility of some of these being due to the impact of terlipressin alone rather than renal improvement alone.

Small sample size, particularly with urine assays, limited statistical power and may have led to false-negative findings. All samples were taken from a single time point—at the time of terlipressin (or placebo) initiation. While the initiation of terlipressin is a valid moment to assess for objective predictors of treatment response, serial sample analysis may have provided additional valuable information. However, longitudinal MAP data over 3 days showed that HRS reversal and MAP increase were linked.[39] Moreover, the rate of ACLF-3 was similar across those who experienced HRS reversal or not. Therefore, specific subanalyses for ACLF-3 and MAP vis-à-vis biomarkers were not performed. It should be noted that all 5 PT patients who achieved HRS reversal were male. Unfortunately, this prevented statistical adjustment for sex and warrants further cautious interpretation of the results in the PT subgroup.

In conclusion, SCr, cystatin C, angiopoietin-2, and beta-2 microglobulin were associated with HRS reversal. Protein and metabolite signals centered on microbiome function and uremic toxins appeared more robust in PT patients and may select for a subgroup that could achieve HRS reversal without terlipressin. Use of novel biomarkers may enrich for terlipressin response but require further external validation for eventual use in clinical practice using larger prospective studies in cohorts with HRS, with and without ACLF.

DATA AVAILABILITY STATEMENT

Discussion of statistical endpoints and analysis is included in the manuscript. Summary aggregate (basic) results (including information on adverse events) and the study protocol are available on clinicaltrials.gov (NCT02770716) when required by regulation. Individual de-identified patient data will not be disclosed. Requests for additional information should be directed to the trial sponsor at medinfo@mnk.com.

AUTHOR CONTRIBUTIONS

Drafted manuscript: Andrew S. Allegretti and Jasmohan S. Bajaj. Guarantor: Jasmohan S. Bajaj. Study design: Andrew S. Allegretti, Josh Levitsky, Pratima Sharma, and Jasmohan S. Bajaj. Statistical analysis: Tianqi Ouyang and Scott Silvey. Interpretation of results: Andrew S. Allegretti, Josh Levitsky, Pratima Sharma, Tianqi Ouyang, Khurram Jamil, Scott Silvey, and Jasmohan S. Bajaj. All authors read and approved the final version of the manuscript. Josh Levitsky is listed posthumously.

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CONFLICTS OF INTEREST

Andrew S. Allegretti reports consultant fees from Mallinckrodt Pharmaceuticals, Ocelot Bio, Motric Bio, Sequana Medical, Bioporto, Acta Pharmaceuticals, as well as DSMB membership for AstraZeneca. The institution of Jasmohan S. Bajaj received grants from Mallinckrodt, Grifols, Bausch, and Sequana. Khurram Jamil is employed and owns stock in Galectin and was previously employed by Mallinckrodt. The remaining authors have no conflicts to report.

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